Appl. No. 10/700,239
Atty. Docket No.: 56029-65456

PATENT

<u>Listing of claims</u>:

1. (previously presented) A method for detecting the presence, absence, or quantity of a segmented negative strand RNA virus in a biological sample suspected of comprising the virus, the method comprising:

    a) providing a genetically engineered vertebrate cell comprising a recombinant RNA molecule that comprises an artificial segment of a segmented negative strand RNA virus or the complement thereof, the artificial segment comprising a 5' untranslated region (UTR) of a segmented negative strand RNA virus, a reporter gene encoding a polypeptide, and a 3' UTR of a segmented negative strand RNA virus, wherein expression of the polypeptide depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus suspected of being comprised by the sample and wherein the cell lacks at least one nucleocapsid protein of the virus;

    b) contacting the cell with a biological sample suspected of comprising a segmented negative strand RNA virus; and

    c) detecting the absence, presence, or quantity of the polypeptide encoded by the reporter gene, thereby indicating the absence, presence, or quantity, respectively, of the virus suspected of being comprised by the sample.

    2. (original) The method according to claim 1, wherein the segmented negative strand RNA virus is selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.

    3. (previously presented) The method according to claim 1, wherein at least one of the 5' UTR and the 3' UTR is a UTR of the virus suspected of being comprised by the biological sample.

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4. (original) The method according to claim 3, wherein the artificial segment comprising the 5' UTR comprises the 5' UTR of the NP segment of an influenza A virus.

5. (original) The method according to claim 3, wherein the 3' UTR of the artificial segment comprises the 3' UTR of the NP segment of an influenza A virus.

6. (previously presented) The method according to claim 1, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of chloramphenicol acetyl transferase, β-galactosidase, β-glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

7. (original) The method of claim 6, wherein the polypeptide encoded by the reporter gene is selected from the group consisting of a green fluorescent protein and a firefly luciferase.

8. (original) The method according to claim 1, wherein the detecting the absence, presence, or quantity of the polypeptide encoded by the reporter gene comprises detecting the absence, presence, or quantity of a photonic signal emitted by the polypeptide.

9. (original) The method according to claim 1, wherein the genetically engineered vertebrate cell is selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRC5 cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

10. (previously presented) A method for detecting the presence, absence, or quantity of a segmented negative strand virus in a biological sample suspected of comprising the segmented negative strand RNA virus, the method comprising:

a) providing a genetically engineered vertebrate cell comprising a recombinant DNA molecule that comprises in 5' to 3' order:

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a promoter for a DNA-dependent RNA polymerase;

a transcription initiation site for the DNA-dependent RNA polymerase;

a DNA sequence encoding an artificial segment comprising a cDNA of a 5' untranslated

region (UTR) of a segmented negative strand RNA virus, a reporter gene encoding a

polypeptide, and a cDNA of a 3' UTR of a segmented negative strand RNA virus; and

a transcription termination site,

wherein said promoter, said transcription initiation site, said artificial segment, and said

transcription termination site are operably linked, and wherein expression of the polypeptide

depends upon the presence in the cell of an RNA-dependent RNA polymerase of the virus

suspected of being comprised by the sample and wherein the cell lacks at least one nucleocapsid

protein of the virus;

b) contacting the cell with a biological specimen suspected of comprising the virus; and

c) detecting the absence, presence, or quantity of the polypeptide encoded by the reporter

gene, thereby indicating the absence, presence, or quantity, respectively, of the virus suspected of

being comprised by the sample.

11. (canceled)

12. (previously presented) The method according to claim 10, wherein the promoter for a

DNA-dependent RNA polymerase is a promoter for RNA polymerase I.

13. (previously presented) The method according to claim 10, wherein the transcription

initiation site is a transcription initiation site for RNA polymerase I.

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14. (previously presented)   The method according to claim 10, wherein the DNA sequence encoding an artificial segment is operably linked to the promoter such that a transcript of the artificial segment is in an anti-sense orientation.

15. (previously presented)   The method according to claim 10, wherein the DNA encoding an artificial segment is operably linked to the promoter such that a transcript of the artificial segment is in a sense orientation.

16. (previously presented)   The method according to claim 10, wherein at least one of the 3' UTR and the 5' UTR is a UTR of the virus suspected of being comprised by the biological sample.

17. (previously presented)   The method according to claim 10, wherein the at least one of the 3' UTR and the 5' UTR is a UTR of the NP segment of an influenza A virus.

18. (original)   The method according to claim 10, wherein the segmented negative strand virus is a virus selected from the group consisting of influenza A virus, influenza B virus, and influenza C virus.

19. (previously presented)   The method according to claim 10, wherein the reporter gene encodes a polypeptide selected from the group consisting of chloramphenicol acetyl transferase, β- galactosidase, β-glucuronidase, renilla luciferase, firefly luciferase, a green fluorescent protein, and secreted alkaline phosphatase.

20. (previously presented)   The method of claim 19, wherein the polypeptide is selected from the group consisting of a green fluorescent protein and a firefly luciferase.

21. (previously presented)   The method according to claim 10, wherein the transcription termination site is an RNA polymerase I transcription termination site.

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22. (previously presented)   The method according to claim 10, wherein the transcription termination site comprises a sequence encoding a self-cleaving ribozyme.

23. (original)   The method according to claim 10, wherein the genetically engineered vertebrate cell is a stably transfected genetically engineered vertebrate cell.

24. (original)   The method according to claim 10, wherein the genetically engineered vertebrate cell is a transiently transfected genetically engineered vertebrate cell.

25. (original)   The method according to claim 10, wherein the genetically engineered vertebrate cell is a transfected cell selected from the group consisting of a 293T kidney cell, an LA-4 cell, a MRCS cell, an A549 cell, a CV-1 cell, a Vero cell, a LLC-MK1 cell, a HEp2 cell, a 2fTGH cell, a U3A cell, a BHK cell, a monkey primary kidney cell, and a Chinese hamster ovary cell.

26.-94. (cancelled)

95. (previously presented)   The method according to claim 1, wherein said genetically engineered vertebrate cell of step (a) lacks all nucleocapsid proteins of the virus.

96. (currently amended)   The method according to claim 1, wherein said genetically engineered vertebrate cell of step (a) lacks at least one viral ~~nucleocapsid~~ protein selected from the group consisting of PA, PB1, PB2 and NP.

97. (previously presented)   The method according to claim 10, wherein said genetically engineered vertebrate cell of step (a) lacks all nucleocapsid proteins of the virus.

98. (currently amended)   The method according to claim 10, wherein said genetically engineered vertebrate cell of step (a) lacks at least one viral ~~nucleocapsid~~ protein selected from the group consisting of PA, PB1, PB2 and NP.